

Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy

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Keywords: melanoma, ipilimumab, abscopal, radiotherapy, expanded access, combination

Abbreviations: ALC, absolute lymphocyte count; CI, confidence interval; CR, complete response; CTLA-4, cytotoxic T-cell lymphocyte antigen-4; EAPs, expanded access programmes; irPR, immune-related partial response; irRC, immune-related response criteria; irSD, immune-related stable disease; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival; PR, partial response; RT, radiotherapy; SD, stable disease; SRT, stereotactic radiotherapy; WBRT, whole-brain radiotherapy

Cancer radiotherapy (RT) may induce what is referred to as the 'abscopal effect,' a regression of non-irradiated metastatic lesions distant from the primary tumor site directly subject to irradiation. This clinical response is rare, but has been surmised to be an immune-mediated phenomenon, suggesting that immunotherapy and RT could potentially synergize. Here, we report the outcome of patients with advanced melanoma treated with the immune checkpoint blockade monoclonal antibody antagonist, ipilimumab followed by RT. Patients were selected for enrollment at the National Cancer Institute 'Fondazione G.Pascale' through the expanded access program in Italy. Those who experienced disease progression after ipilimumab thus received subsequent RT and were selected for analysis. Among 21 patients, 13 patients (62%) received RT to treat metastases in the brain and 8 received RT directed at extracranial sites. An abscopal response was observed in 11 patients (52%), 9 of whom had partial responses (43%) and 2 had stable disease (10%). The median time from RT to an abscopal response was 1 month (range 1–4). Median overall survival (OS) for all 21 patients was 13 months (range 6–26). Median OS for patients with abscopal responses was extended to 22.4 months (range 2.5–50.3) vs. 8.3 months (range 7.6–9.0) without. A local response to RT was detected in 13 patients (62%) and, of these, 11 patients (85%) had an abscopal response and abscopal effects were only observed among patients exhibiting a local response. These results suggest RT after ipilimumab may lead to abscopal responses in some patients with advanced melanoma correlating with prolonged OS. Our data also suggest that local responses to RT may be predictive of abscopal responses. Further research in larger randomized trials is needed to validate these results.

Introduction

Malignant melanoma is among the most aggressive cancers, with a strong tendency to metastasize early in the disease course. In fact, until relatively recently, approved treatment options for patients with metastatic melanoma have been extremely limited. However, advances in our understanding of tumor

biology have led to the exploration of novel treatment strategies aimed at containing or eradicating systemic disease. At the forefront of this cutting-edge research has been the development of ipilimumab, a monoclonal antibody that targets the inhibitory immune checkpoint regulator cytotoxic T-cell lymphocyte antigen-4 (CTLA-4). Ipilimumab has been shown to significantly improve overall survival (OS) of melanoma patients in comparison

to the standard-of-care treatment across the disease spectrum.^{1,2}

A key focal point of ongoing research into novel therapeutic regimens for advanced melanoma is how to maximise the clinical benefit of conventional treatments. Increasing evidence suggests that immunotherapy may have additive, or possibly even synergistic, effects when used in combination with other treatments.³ For example, potential

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Submitted: 01/23/2014; Revised: 03/24/2014; Accepted: 04/03/2014; Published Online: 05/14/2014

Citation: Grimaldi AM, Simeone E, Giannarelli D, Muto P, Falivene S, Borzillo V, Giugliano FM, Sandomenico F, Petrillo A, Curvietto M, et al. Impact of radiotherapy in patients with advanced melanoma who progressed after ipilimumab 3 mg/kg. *Oncolmunology* 2014; 3:e28780; <http://dx.doi.org/10.4161/onci.28780>

Table 1. Baseline characteristics and treatment. Summary of baseline patient characteristics measured at the start of ipilimumab therapy and details of treatment received

Characteristic	Patients receiving RT after ipilimumab	All ipilimumab-treated patients
Total number of patients	21	120
Median age, years (range)	58 (21–77)	58 (18–86)
Male/female, n (%)	11 (52)/10 (48)	60 (50)/60 (50)
M stage, n (%)		
M0 Stage III	0	9
M1a	2 (10)	4
M1b	2 (10)	6
M1c	17 (81)	101
LDH level, median (range)	480 (223–905)	490 (190–1816)
Time from diagnosis to ipilimumab, months (range)	35 (2–144)	35 (2–182)
BRAF status, n (%)		
Mutated	3 (15)	31 (26)
Wild-type	18 (85)	84 (70)
Unknown	0	5 (4)
Number of previous therapies, n (%)		
1	20 (95)	101 (84)
2	1 (5)	19 (16)
Previous therapy type, n (%)		
Cisplatin + temozolomide	5 (24)	55 (46)
Dacarbazine	8 (38)	40 (33)
Fotemustine	2 (10)	15 (13)
Temozolomide	3 (14)	14 (12)
MAGE A3	1 (5)	3 (3)
MEK 162	1 (5)	3 (3)
Dabrafenib	1 (5)	6 (5)
Vemurafenib	0	12 (10)
Time to progression from ipilimumab, months (range)	4 (3–6)	5 (4–6)
Ipilimumab cycles		
4 ^a	20 ^a (95)	97 ^b (81)
3	1 (45)	11 (9)
2	0	10 (8)
1	0	2 (2)
Time from ipilimumab to RT, months (range)	5 (4–8)	–
RT site		
Brain	13 (61)	–
WBRT	9 (69)	–
SRT	4 (31)	–
Bone	4 (19)	–
Metastatic distant lymph nodes	2 (10)	–
Cutaneous metastases	2 (10)	–

^a1 patient received a further 4 cycles of ipilimumab as retreatment.; ^b11 patients received a further 4 cycles of ipilimumab as retreatment. Abbreviations: LDH, lactate dehydrogenase; RT, radiotherapy; SRT, stereotactic radiotherapy; WBRT, whole-brain radiotherapy.

additive effects of radiotherapy (RT) and immunotherapy may explain several reports of distal anticancer effects to RT termed ‘abscopal’ responses in advanced melanoma patients receiving palliative RT in addition to ipilimumab treatment.⁴⁻⁶ The ‘abscopal’ effect can be broadly defined as a reaction outside an irradiated area but within the same organism.⁷ This phenomenon has been reported to occur in the treatment of a variety of malignancies, including hepatocellular carcinoma, lymphoma, and melanoma.^{5,6,8-10} Despite preclinical evidence that the abscopal effect may be mediated by radiation-induced immune responses,¹¹ reports of abscopal responses in clinical practice have been infrequent, suggesting RT alone may be insufficient to induce a general systemic and robust antitumor effect. However, experimental data from murine tumor models suggest that coupling irradiation with immunotherapy could amplify the radiation-induced immune response sufficiently to elicit an abscopal effect.^{12,13}

Understanding the potential benefits of combination therapies is essential to the design of optimized treatment strategies tailored to advanced melanoma patients. To date, only a small number of early-phase trials have investigated the combination of RT and immunotherapy in a clinical setting. Information on the potential benefits of combining RT and ipilimumab in patients afflicted with advanced melanoma are currently limited to a few case reports, and as of yet, no data are available from prospective trials. Case series, including retrospective analyses of data from expanded access programmes (EAPs), can provide additional insights into the clinical benefits of this combinatorial therapy. Here, we mined such data through the EAP in Italy to examine whether melanoma patients with progressive disease following 3 mg/kg ipilimumab and subsequently treated with localized RT exhibited abscopal anticancer effects. We further explored whether observations of local responses to RT could potentially be used as a clinical marker predictive of patients most likely to exhibit an abscopal response.

Table 2. Responses and survival following RT in patients who progressed after ipilimumab. Local and systemic (abscopal) responses to radiotherapy (RT) after progression with ipilimumab and median overall survival (OS) of patients stratified according to the presence or absence of abscopal response

Treatment outcome	
Median PFS with ipilimumab, months (range)	4 (2.3–6)
Local response to RT	
Yes	13 (62)
No	8 (38)
Immune-related response after RT (abscopal response)	
Abscopal irPR	9 (43)
Abscopal irSD	2 (10)
No abscopal response	10 (48)
Time from RT to abscopal response, months (range)	1 (1–4)
Median follow-up, months (range)	11 (6–32)
Median OS, months (95% CI)	
All patients	13 (6–26)
Patients with an abscopal response	22.4 (2.5–50.3)
Patients without an abscopal response	8.3 (7.6–9.0)
<i>P</i> value OS +/- abscopal response	0.002

Abbreviations: CI, confidence interval; irPR, immune-related partial response; irSD, immune-related stable disease; OS, overall survival; PFS, progression-free survival; RT, radiotherapy.

Results

Melanoma patient demographics and treatment course

Of the 21 patients who received localized RT after ipilimumab treatment, 18 had wild-type BRAF and 3 had mutant BRAF^{V600} subtypes of melanoma (recorded retrospectively). Twenty of the 21 patients had received a single line of therapy for advanced melanoma prior to ipilimumab treatment and 1 patient had received 2 previous systemic therapies. Baseline patient and disease characteristics were similar to those among all patients treated in the Italian EAP at the National Cancer Institute. Twenty patients (95%) completed all 4 cycles of ipilimumab therapy and, among these, 1 patient was retreated with a second course, receiving a further 4 cycles of ipilimumab therapy. The remaining patient received only 3 doses of induction therapy due to the sudden onset of symptoms secondary to the brain metastases. The median time to progression from the first dose of ipilimumab treatment was 4 mo (range 2.3–6) and the median time from the

start of ipilimumab treatment to RT was 5 mo (range: 3.4–8). The study population included 13 patients (62%) receiving RT to treat brain metastases, among which were 9 patients who received whole-brain radiotherapy (WBRT) and 4 patients who received stereotactic radiotherapy (SRT). The remaining 8 patients (38%) received RT directed at bone (*n* = 4; 2 femoral and 2 vertebral), lymph node (*n* = 2), and cutaneous (*n* = 2 at chest wall) metastases. Baseline patient characteristics measured at the start of ipilimumab therapy and treatment parameters are shown in Table 1.

Response to RT

Irradiation-induced tumor responses are summarized in Table 2. Among the 21 patients receiving RT due to disease progression after initial ipilimumab treatment, 13 (62%) exhibited a local response (response at the site directly treated with RT) whereas the remaining 8 patients (32%) did not display a local response to RT. An immune-related tumor-inhibitory response outside of the irradiated site (i.e., an abscopal response) was observed in 11 patients (52%).

Table 3. Type and site of response in patients with abscopal responses. Details of the site and dose of locoregional RT, and type of abscopal response observed in distant lesions

Patient, #	RT site (type)	RT dose, Gy/fractions	Response	Site of abscopal response (distant target lesions)
1.	Brain (WBRT)	30/10	PR	Liver metastases
2.	Brain (WBRT)	30/10	PR	Pelvic relapse
3.	Chest wall (cutaneous relapse) + right axilla	50/25	PR	Liver and cutaneous metastases
4.	Right inguinal lymph node	20/5	PR	Gastric, cutaneous, lung, lymphnodal and retroperitoneal abdominal metastases
5.	Brain (WBRT)	30/10	PR	Liver, bilateral axillary and right ovarian metastases
6.	Brain (WBRT)	30/10	PR	Lung, cutaneous, lymphnodal and abdominal metastases
7.	Right chest wall (cutaneous relapse)	30/10	SD	Lymphnodal, cutaneous and chest wall metastases
8.	Vertebral metastasis	30/10	SD	Lung metastases
9.	Brain (SRT)	24/1	PR	Cutaneous metastases
10.	Brain (SRT)	20/1	PR	Liver metastases
11.	Brain (SRT)	24/1	PR	Lung metastases

The responses reported in the table are the systemic responses; however, all 11 patients with an abscopal response also had a local response to RT. Abbreviations: PR, partial response; RT, radiotherapy; SD, stable disease; SRT, stereotactic radiotherapy; WBRT, whole-brain radiotherapy.

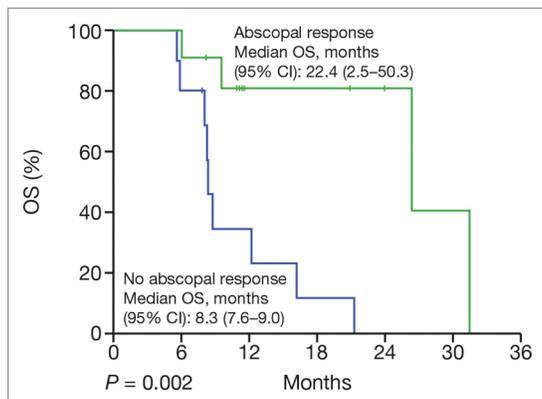


Figure 1. Patient survival according to abscopal responses. Kaplan–Meier curves depicting overall survival (OS) curves among patients who received RT after progression with ipilimumab, according to the presence or absence of an abscopal response (present in 11 patients and absent in 10 patients). Groups were compared using the log-rank test; $P = 0.002$.

These included 9 patients (43%) with an abscopal partial response (PR) and 2 patients (10%) with stable disease (SD) of more than 3 mo (Table 3). Abscopal responses were observed in 23 metastatic sites in total, of which 11 (47.8%) were in metastatic lesions of soft tissue or lymph nodes. The median time from RT to an abscopal response was 1 mo (range 1–4). Abscopal effects were exclusively observed among patients who also displayed a local response to RT and 11/13 (85%)

of the patients with a local response exhibited an immune-related abscopal response to RT (Table 2 and Table 3). Local tumor responses in these 11 patients, included 7 in brain metastases, 2 in cutaneous metastases, 2 in metastatic lymph nodes, 1 in a vertebral metastasis and 1 in a femoral metastasis. Median time to assessment of disease after the completion of RT was 40 d (range 30–60). An abscopal effect was absent from 2 of the patients who achieved a local response to RT, one of whom developed SD after RT of a femoral metastasis and another with PR after RT of a metastatic lymph node in the groin. Both

of these patients were diagnosed with stage M1c melanoma 1 with mucosal melanoma of the glans and displaying elevated lactate dehydrogenase levels (LDH), and 1 with acral melanoma of the heel with normal LDH levels. Both patients were BRAF negative and had completed the ipilimumab treatment regimen of 4 cycles of second-line ipilimumab for metastatic disease. No additional parameters analyzed in patients with or without an abscopal

response were identified as potentially predictive of an abscopal effect, other than the presence of a local response to RT. However, despite both patient groups having a relatively similar median absolute lymphocyte count (ALC) at baseline (20 200/ μL vs. 17 800/ μL ; $P = 0.32$), a larger increase in median ALC during ipilimumab induction therapy was noted in patients with an abscopal response vs. those without an abscopal response, such that the static value at week 10 was significantly ($P = 0.001$) higher in those with an abscopal response (20 273/ μL) vs. without (13 222/ μL). The median ALC pre-RT was also significantly higher in patients with an abscopal response than in those without (18 900/ μL vs. 12 833/ μL ; $P = 0.04$); however, this difference did not remain statistically significant after RT (12 444/ μL vs. 8,750/ μL ; $P = 0.16$), possibly due to a decrease in the number of patients for whom ALC data were available.

With a median follow-up of 11 mo (range 6–32 mo), median OS for all 21 patients included in the analysis, measured from the start of ipilimumab treatment, was 13 mo (within a 95% CI ranging from 6–26 mo; Table 2). Median OS for patients with an abscopal response was significantly longer than for patients who did not have an abscopal response

(Fig. 1; $P = 0.002$). Median OS was 22.4 mo (within a 95% CI ranging from 2.5–50.3 mo) vs. 8.3 mo (within a 95% CI ranging from 7.6–9.0 mo) for the 2 groups, respectively (Fig. 1; Table 2). Representative cases of patients in this study exhibiting abscopal responses are shown in Figure 2 and Figure 3. Of all 120 patients treated with ipilimumab at the National Cancer Institute, median OS among 50 patients who had progressed with ipilimumab at the first assessment but did not receive RT was 5.8 mo (within a 95% CI ranging from 3.5–8.1 mo) and this was not significantly different from that observed in patients who received RT but did not have an abscopal response ($P = 0.27$).

Discussion

Preclinical and clinical evidence suggests that RT may enhance the cancer therapeutic benefit of ipilimumab although, to date, no data are available from randomized clinical trials to determine whether these 2 treatment approaches have synergistic antitumor effects.^{4-6,13-15} This is the first case series (and largest cohort to date) describing the abscopal effect elicited by sequential treatment with ipilimumab and RT in patients with advanced melanoma. All of the patients included in the analysis had received systemic therapy prior to ipilimumab treatment and most were BRAF wild-type, meaning that third-line treatment options were extremely limited and RT was the only standard-of-care option available. The abscopal responses occurring in 52% of patients within our retrospective analysis suggest that tumor control could potentially be achieved via RT following progression after ipilimumab-induction therapy or upon retreatment in the event of relapse. Furthermore, median OS for patients with abscopal response was significantly

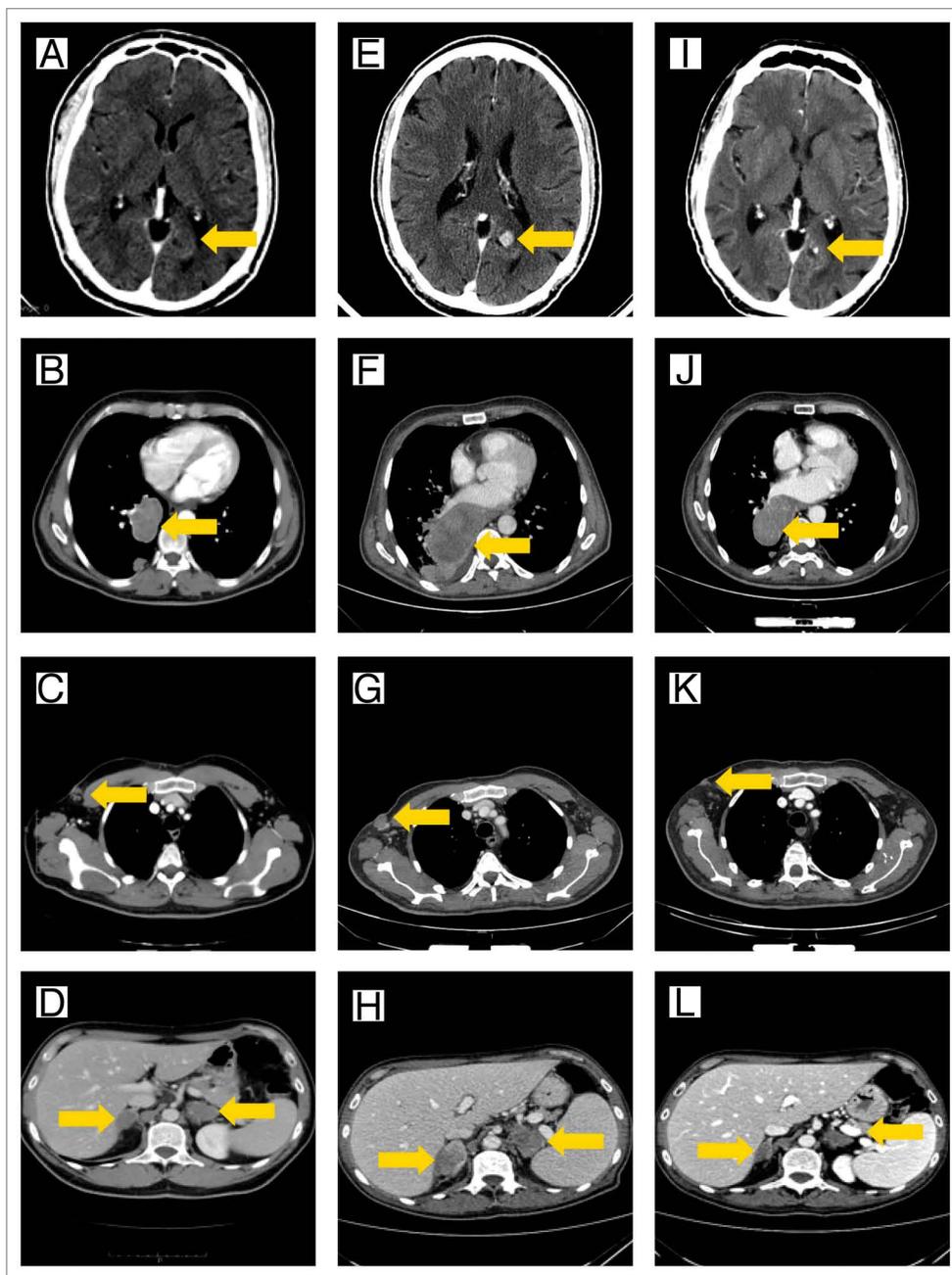


Figure 2. Patient Case 1. (A–L) A 54-y-old male patient received 4 cycles of ipilimumab 3 mg/kg (June to August 2012) followed by palliative whole-brain radiotherapy (WBRT) in September 2012 for symptomatic brain metastases. Whole body 128-slice CT scans (1 mm thickness) were performed at baseline, post-ipilimumab and post-radiotherapy (post-RT). (A–D) Baseline, pre-ipilimumab scans from May 2012. (E–H) Post-ipilimumab scans from September 2012 (pre-RT) showing (E) brain metastasis (1 of the multiple lesions) and progression of lung (F), cutaneous (G) and lymphodal metastases (H) after ipilimumab treatment. (I–L) Post-RT follow-up CT scan (I) from October 2012 showing a local response and reduction of lung (J), cutaneous (K) and lymphodal (L) metastases indicative of abscopal response.

longer compared with patients without an abscopal response, suggesting systemic responses to RT after disease progression following ipilimumab regimens may translate into meaningful improvements in patient survival. Notably, median OS

in the 10 patients without an abscopal response was not significantly different to that observed among 50 patients who had progressed but did not receive RT, arguing against a selection bias for RT as a whole.

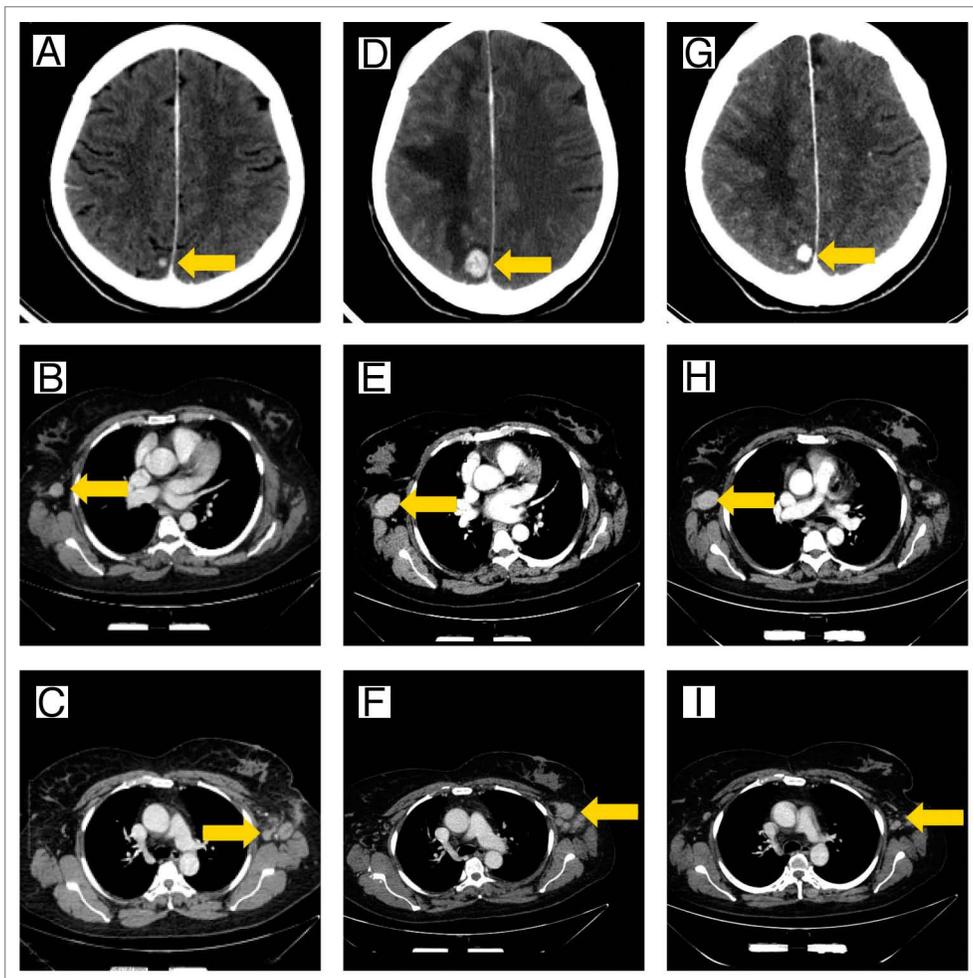


Figure 3. Patient Case 2. (A–I) A 52-y-old female patient received 4 cycles of ipilimumab 3 mg/kg (May to August 2012) followed by palliative whole-brain radiotherapy (WBRT) in August to September 2012 for symptomatic brain metastases. Whole body 128-slice CT scans (1 mm thickness) were performed at baseline, post-ipilimumab and post-RT. (A–C) Baseline, pre-ipilimumab scans from May 2012. (D–F) Post-ipilimumab scans from August 2012 showing representative brain metastasis (D) and lymph nodal (E–F) metastases. (G–I) Post-RT follow-up scan from September 2012 showing a local response (G) and reduction of lymph nodal (H–I) metastases indicative of abscopal response.

A second aim of this analysis was to determine whether local responses to RT could be of use to predict which patients are most likely to achieve an abscopal, and thus most clinically beneficial, response. Of the 13 patients with a local response to RT, 11 (85%) achieved an abscopal PR (i.e., regressed metastatic lesions) or even stable disease. Therefore, local responses may prognosticate abscopal responses to RT in melanoma patients following ipilimumab treatment. Based on the finding that only patients with a local response achieved an abscopal response, we could further speculate that a local response may be a precursor to a systemic, abscopal response, particularly with the

treatment regimen utilized here. However, considering the limited number of patients in our cohort, further investigations are warranted to explore the potential relationship between local anticancer responses and systemic abscopal effects of combinatorial ipilimumab and RT. Similarly, patients with an abscopal response had a significantly higher median ALC after ipilimumab-induction therapy (and preceding RT) than those without an abscopal response, suggesting that lymphocyte counts after ipilimumab treatment could be indicative of patients that are more likely to have a clinically beneficial abscopal effect following subsequent RT. However, on the basis of

this limited study, it is not possible to definitively determine whether ALC is prognostic for abscopal patient responses to combined ipilimumab and irradiation therapy.

Our findings are consistent with prior single case reports of the abscopal effect detected in patients with advanced melanoma receiving ipilimumab and RT.^{5,6} In one such report, the presence of anti-MAGE-A3 antibodies was identified upon serological testing prior to ipilimumab treatment, suggestive of an existent systemic anticancer immune response correlating with the abscopal effect.⁵ Several other reports describe complete responses (CRs), or durable disease control, in patients with either advanced melanoma or non-small cell lung cancer, treated with sequential or concurrent ipilimumab plus RT, providing further rationale for this potent and immunostimulatory therapeutic combination.^{4,14,16-21}

In our study, in addition to the 9 patients with a PR, the 2 patients with immune-related SD were also classed as having an abscopal response since both of these patients experienced prolonged SD, despite having confirmed progressive disease prior to RT. Therefore, it appears likely that RT induced a positive antitumor immunologic effect in these patients as well.

In the present analysis, less than 50% of the responding metastatic sites distal from the irradiated lesions were in soft tissue or lymph nodes. Observations of abscopal effects in diverse metastatic sites may reflect a myriad of distinct mechanisms by which RT could enhance systemic immune responses.²²⁻²⁷ Given the complexity of such interactions, a future challenge in developing combinatorial therapeutic regimens using ipilimumab and RT will be to determine the optimal timing and dosage of either treatment to achieve maximum immunologic and clinical benefit. The results of our analysis, taken together with other

published case reports, suggest that the abscopal effect may occur irrespective of whether RT is given prior to or following ipilimumab treatment.^{5,6} Furthermore, in a retrospective analysis of patients who received SRT with, or without, ipilimumab (administered either before or after SRT), the median OS was markedly extended to 21.3 mo (95% CI, range from 6.43–26.7 mo) for patients who received ipilimumab and SRT bimodal therapy as compared with 4.9 mo (95% CI, range from 3.3–10.4 mo) with SRT alone, and no significant difference in OS according to the initiation of ipilimumab dosing relative to the timing of the first SRT treatment.¹⁴ However, some preclinical data assaying concurrent and (or) sequential treatment suggest that both the timing of anti-CTLA-4 blockade therapy administration relative to RT as well as the type of irradiation dose fractionation can affect the therapeutic efficacy of this combination.^{13,14,28,29} Clinical trials will help to determine the optimal dosage and schedule for combination, or sequential, RT and ipilimumab treatment regimens.

Safety is essential for the development of novel therapeutic approaches, so it should be born in mind that RT can lead to prolonged release of immunogenic molecules, a pathophysiologic response that could potentially increase the risk of inflammatory reactions with ipilimumab. In these regards, a case series of 3 patients treated sequentially with RT followed by 3 mg/kg ipilimumab, and evaluated post-therapy by cerebral magnetic resonance imaging revealed radiation-induced necrosis of the brain at the irradiated sites. Notably, however, all 3 of these patients responded favorably to ipilimumab induction therapy.¹⁹ In another retrospective study, concurrent ipilimumab and RT was not found to be associated with higher than expected rates of immune-related adverse events.³⁰ Our retrospective study here focused on abscopal responses and subsequent survival outcomes and did not include an investigation of the safety of RT implementation after ipilimumab treatment and disease progression. However, we anticipate that the results of ongoing trials will elucidate potential

safety issues with combined or sequential ipilimumab and RT treatments.

As the first case series describing abscopal effects induced by RT following ipilimumab therapy in advanced melanoma patients with progressive disease, this report provides valuable insight into the potential benefits of combined immunotherapy and RT in such patients. However, these preliminary data should be interpreted with caution and any conclusions should be tested in an independent case series. The small number of patients included in our analysis limits the strength of statistical comparisons. Thus, the significance of any potential survival benefit of abscopal effects elicited by RT after ipilimumab treatment in patients with progressive, late-stage disease is unclear. Likewise, any conclusions regarding the value of local responses to RT in predicting abscopal effects are purely speculative as the analysis is confounded by variation in other factors that may affect treatment outcomes. Detailed analysis from a much larger patient cohort is needed to allow correction for variables such as LDH levels, site(s) of metastases, dose of RT and the number of ipilimumab doses received, all of which are confounding factors that may impact outcome. The abscopal effect is generally considered a rare phenomenon and has so far only been reported in a limited number of patients receiving ipilimumab and RT therapy. Nevertheless, in our analysis, we found that more than half of the 21 patients treated with RT after receiving ipilimumab experienced distal tumor regression consistent with an abscopal effect. Although we cannot rule out the possibility that at least some of the systemic responses observed were unrelated to RT, the incidence of delayed responses was much higher than would be expected with ipilimumab treatment alone.

Finally, to provide further support for the potential synergy of ipilimumab and RT, it would be worthwhile to compare the results from this analysis with data from patients treated with RT following disease progression after other treatment modalities. The abscopal effect has been reported previously in an advanced

melanoma patient who received RT after treatment with the BRAF inhibitor vemurafenib, which may have resulted from increased immunogenicity against melanoma cells following the BRAF inhibitor treatment.³¹ However, the potential for abscopal responses may be greater when RT is combined with immunotherapies, such as ipilimumab, since their mechanism of action is primarily immune-based.

In conclusion, our data suggest that administering RT after progression with ipilimumab may lead to abscopal effects in some patients and that these responses appear to be associated with prolonged survival. This treatment sequence may therefore represent a new therapeutic strategy in the treatment of metastatic melanoma. Well-designed clinical trials seeking to standardize the dose and scheduling of RT are needed to confirm these results and shed light on the anticancer immunostimulatory role of RT applied in concert with ipilimumab.

Material and Methods

Melanoma patients

This was a retrospective analysis of 21 patients with advanced melanoma who progressed after receiving ipilimumab and were subsequently treated with locoregional RT. These patients were among those enrolled in the ipilimumab EAP at the National Cancer Institute 'Fondazione G. Pascale' in Naples, Italy (n = 120). Physicians were able to request ipilimumab via the EAP for patients who had no alternative treatment option available and who met all of the EAP inclusion criteria as previously described.³²⁻³⁶ The EAP was approved by a local ethics committee, and all participating patients provided signed informed consent before enrollment and according to the Declaration of Helsinki. Patients with disease progression following initial ipilimumab therapy received subsequent RT to localized brain or extracranial (bone, lymph node, cutaneous or vertebral) metastases, comprising the 21 patients retrospectively assessed in this study.

Immunotherapy, radiotherapy, and tumor-response assessments

Ipilimumab at a dosage of 3 mg/kg was administered intravenously every 3 wk for 4 doses. Tumour assessments were conducted by the investigators at baseline, week 12 and every 12 wk thereafter using immune-related response criteria (irRC), as previously described.³⁷ Patients who achieved immune-related disease control after induction therapy but who later progressed were eligible for retreatment with ipilimumab. Patients classified as having immune-related progressive disease (i.e., an increase in tumor burden of $\geq 25\%$ relative to the minimum recorded tumor burden) and for whom no other therapeutic option was available were eligible for RT and all patients so treated were included in this analysis. All patients with extracranial lesions received 3D conformal RT for symptom palliation (bleeding or pain) using one of the following schedules: 20 Gray (Gy) delivered in 5 fractions; 30 Gy delivered in 10 fractions (for bone and nodal metastases); 30 Gy in 10 fractions or 50 Gy in 25 fractions (for cutaneous lesions). Patients with multiple brain metastases underwent whole-brain RT (WBRT) at a total dose of 30 Gy in 10 fractions. In patients with 1–3 brain metastases (≤ 4 cm maximum dimension), stereotactic RT (SRT) using the LINAC-based stereotactic system was provided at the dose of 20 Gy or 24 Gy in a single fraction. A simulation planning-CT scan was acquired for all patients at variable slice thickness (3–5 mm) depending on the site of disease, using a customised immobilisation system (i.e., thermoplast mask for brain). Local and abscopal responses to RT were defined according to irRC. Local response to RT was defined as dimensional reduction of the irradiated lesion as evaluated by CT scan performed 40 d after completion of RT. An abscopal response to RT was defined as the dimensional reduction of metastases outside the irradiated area. The abscopal effect was assessed by total body CT scan performed 40 d after completion of RT.

Lactate dehydrogenase (LDH) levels and absolute lymphocyte count (ALC) were evaluated in peripheral blood and

sera samples collected at baseline, weeks 4, 7, and 10 of ipilimumab therapy and pre- and post-RT.

Statistical analysis

Patient and disease characteristics were analyzed using descriptive measures and expressed as relative frequencies (percentages) for discrete variables, and median and range for continuous variables. OS was estimated using Kaplan–Meier analysis and expressed as a median value with corresponding 2-sided 95% confidence interval (CI). Statistical significance of differences in survival curves between patients with or without an abscopal response was calculated using the log-rank test.

Disclosure of Potential Conflicts of Interest

P.A.A. has/had advisory or consultant role for Bristol–Myers Squibb, Roche–Genentech, GlaxoSmithKline, and Novartis. He received research funding from Bristol–Myers Squibb. He received honoraria from Bristol–Myers Squibb, Roche–Genentech, and GlaxoSmithKline. E.S. received honoraria from Bristol–Myers Squibb. All the other authors have no conflicts of interest to declare.

Acknowledgments

The EAP was sponsored by Bristol–Myers Squibb (BMS). Editorial and writing assistance was provided by StemScientific, funded by BMS. Statistical support was provided by Clinical Research Services, funded by BMS. The authors would like to thank the patients and investigators who participated in the European EAP.

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